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14. ABSTRACT

This SBIR project aims to develop hyperimmune human polyclonal antibody that neutralizes the toxic activity of staphylococcal enterotoxin B (SEB) as treatment for toxic shock induced by SEB disseminated as aerosol in a biowarfare scenario. The primary goal of Phase I was to demonstrate the feasibility of therapeutic intervention with immunoglobulin enriched with antibodies against SEB. Feasibility of the approach was demonstrated in cellular assays and a mouse model of SEB induced toxic shock. IVIG and specific human anti-SEB antibodies purified from IVIG was used as a surrogate for hyperimmune globulin. It was demonstrated that human polyclonal antibodies can potently inhibit the toxicity of SEB. Antibodies were also able to protect mice from lethal challenge with SEB. Several cell based assays and respective SOPs were developed for testing the neutralizing activity of human polyclonal antibodies toward SEB. These assays will be used in Phase II to test the activity of sera from individuals vaccinated with IBT's recombinant SEB vaccine (STEBVax). Immunoglobulin will be purified from plasmapheresed vaccinated individuals to conduct preclinical efficacy studies in animal models of SEB toxic shock. It is anticipated that the Phase II project will result in a strong candidate for clinical development.

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Introduction

This SBIR project addresses the need to develop a treatment for toxic shock induced by staphylococcal Enterotoxin B (SEB), which, in addition to a current public health threat, may be disseminated as an aerosol in a biowarfare scenario. Specifically, we seek to develop a hyperimmune human polyclonal antibody that neutralizes the toxic activity of SEB. The primary goal of Phase I was to demonstrate the feasibility of both protection and therapeutic intervention using immunoglobulin enriched for antibodies against SEB. A secondary goal was to develop and optimize assays for evaluation of the therapeutic polyclonal human antibodies and to develop the required SOPs. Both goals have been achieved.

Body

Phase I Objective 1: *Assemble a cohort of monkey sera from IBT's prior vaccine studies and intravenous immunoglobulin (IVIG) preparations from Omrix Biopharmaceuticals.*

Twenty eight IVIG samples (0.5 gram each) and 30 serum samples (2 ml each) from monkeys vaccinated either with adjuvant, or two different doses of STEBVax were collected. The monkey sera originated from a concurrent GLP-safety study for STEBVax in which monkeys received four injections of STEBVax at 50 and 200 μg. However upon assay, the rhesus samples indicated an assay artifact in which even the control animals showed an increase in IFN-g using our assay conditions. Rather than investigate and determine the exact cause of the artifact, we were able to obtain 30 plasma samples from individual human donors from Omrix. These samples did in fact have intrinsic anti-SEB levels and were used most effectively for assay development and subsequent studies. The IVIG samples were aliquoted and stored at 4°C as instructed by Omrix, plasma and sera were stored at -20°C until use.

Phase I Objective 2: *Test human IVIG preparations and identify a short list of high titer, and purified anti-SEB human antibody samples. Confirm the titer of monkey immune sera.*

All the IVIG, human plasma, and monkey sera samples were tested by ELISA for reactivity to SEB using an endpoint dilution method. All of the control monkey sera showed titers below 1:100. Both vaccinated groups, regardless of dose, showed high titers of anti-SEB antibodies, ranging mostly from 1:10,000 to 1:100,000. The IVIG batches were fairly similar with respect to anti-SEB content, reflecting the fact that they were pooled and purified from a large number of donor plasmas. Eight IVIG samples were selected with relatively high, medium, and low levels of anti-SEB content. This combination of samples was selected to allow *in vitro* and *in vivo* activity to be correlated with the specific anti-SEB content of the IVIG samples. The titers of these eight IVIG samples as determined by ELISA are shown in ***Table B.1***.

IVIG#	anti-SEB μg/g IVIG
J 16G171	331.0
J 29G280	428.5
J 15G165	345.5
I 42335	315.0
I 36291	364.2
I 28402	395.9
I 19135	330.8
I 49395	374.2

Table B.1: Anti-SEB content of tested IVIG samples (μg specific antibody per gram of IVIG).

The mean anti-SEB content of the IVIGs was 361±38 μg/g. The median was virtually identical to the mean (355 μg/g), suggesting a normal distribution as expected. Overall, the levels of anti-SEB were fairly low. This is most likely because the titers are the result of natural exposure to SEB rather than specific vaccination and thus represent the naïve population. Based on these data, even a very high dose of IVIG (2 g/kg) would provide a 70 kg patient about 40-50 mg of anti-SEB antibody. Considering that only a fraction of these antibodies are actually neutralizing SEB, the effective dose would be reduced to low mg amounts – amounts that most likely would be insufficient to counteract the activity of disseminated SEB. *This finding further reinforces the need to develop hyperimmune antibody therapeutics for SEB, as proposed herein.* The titer of IVIG for several other superantigens also was determined. The mean values for anti-toxin content of the eight IVIG batches are shown in ***Figure B.1***.

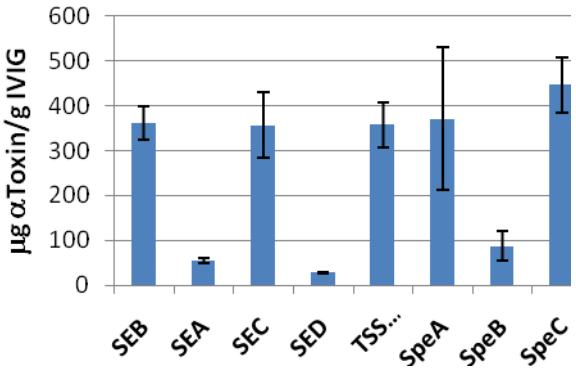

Figure B.1. Mean anti-toxin content of IVIG batches

Since no negative IVIG pool was found, a decision was made to also screen individual plasma unitswith the expectation that both high titer and low titer individuals would be identified. Thus, 30 samples of plasma units (anonymous) from the Omrix production line were tested by SEB capture ELISA. As shown in ***Table B.2***, these samples indeed reflected a wide range of anti SEB titers, from negative to strongly positive. The results were reported to Omrix upon which Omrix produced two batches of IgG from the specified plasma units: one batch was produced from the two highest titer units, and the other batch was produced from the low titer units. The batch produced from the low titer units will be further depleted from anti-SEB antibodies by an affinity column and serve as the negative control for future assays.

IBT#	Omrix#	ELISA Titer (ug/ml):	IBT#	Omrix#	ELISA Titer (ug/ml):
028	FL05774	7.31	019	K856505	0.95
006	K556491	6.72	029	LX17432	0.90
027	LN16512	3.20	004	FL05775	0.81
007	FL05769	2.90	014	FL05780	0.77
005	K560570	2.88	008	LN15821	0.60
001	FH46693	2.35	002	LN16574	0.55
026	FL05791	2.24	011	LN17080	0.54
024	LN15808	2.19	030	FL05782	0.52
023	KT61129	2.03	003	LN15826	0.50
017	K556499	1.97	020	FW39802	0.47
016	KT66334	1.79	022	KT61140	0.37
012	K560544	1.49	021	FL05785	0.33
018	FL05793	1.47	015	FH46676	0.33
010	LN17076	1.19	025	FH46692	0.32

Table B.2. Anti-SEB titers of individual plasma units. The samples are ordered according to anti-SEB titer

Phase I Objective 3: *Establish robust in vitro assays based on neutralization of SEB-induced T cell proliferation and cytokine release and develop SOP for these assays. Standardize and calibrate LPS and SEB for in vivo studies.*

Methods for reliable and quantitative measurement of T cell activation by SEB were developed using human peripheral blood mononuclear cells (PBMC). Several read-outs were tested, including (1) proliferation using both radioactive and non-radioactive techniques and (2) the release of proinflammatory cytokines IFNγ, TNFα, IL-1, and IL-6 using commercially available kits (R&D Systems, Minneapolis, MN). In multiple experiments, it was shown that IFNγ release clearly correlates with other read-outs and provides a reliable, cost effective, and logistically preferred measure of activity. Therefore, this assay was selected as the primary screening assay. A T cell activation assay also was established for other superantigens such as SEA, TSST-1, SED, SEC, SPeA, SPeB, and SpeC. The specific dose ranges for each toxin were determined (***Figure B.2A***). ***Figure B.2B*** shows the inhibition of SEB induced T cell activation by two IVIG batches which are representative of the other OMRIX pooled samples. To ensure that the inhibitory effects of IVIG were specific, the influence of IVIG treatment on phytohemagglutinin (PHA) induced T cell activation was examined: it was shown that IVIG did not inhibit PHA-induced activation (***Figure B.2C***). The assays to determine the inhibitory concentration range for a given dose of toxin have been optimized and SOPs were developed for all 8 toxins described previously.

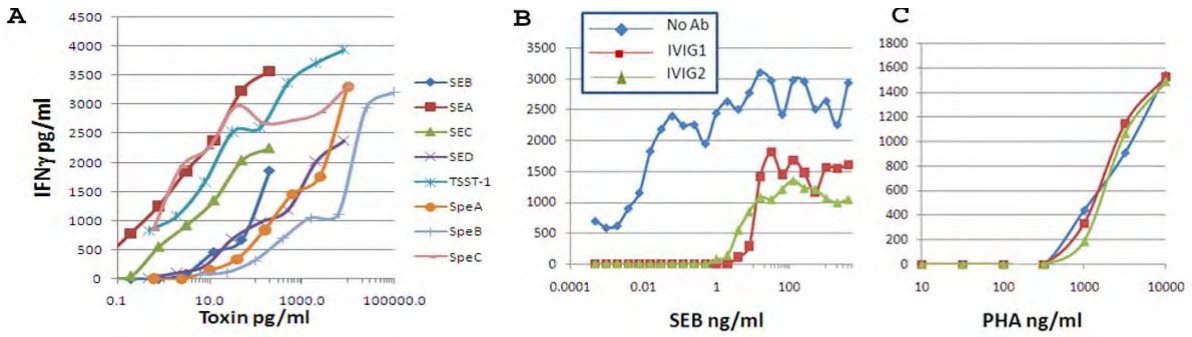

Figure B.2. T-cell activation, as measured by IFNγ release, in response to multiple toxins (A). Toxin neutralization assay of two individual OMRIX pool samples (with control) based on IFNγ release in PBMC (B) compared to PHA activation control (C).

Large batches of SEB (5 mg) and LPS (100 mg) were purchased and aliquoted. Both LPS and SEB were calibrated in two separate mouse challenge experiments. As a result of these experiments, it was shown that 20-40 μg of LPS was sufficient to induce lethality in combination with SEB when given as an IP injection four hours after the initial SEB challenge. These experiments were based on previous experiments and our findings are also consistent with the literature (1). The SEB LD_{50} was calculated from the calibration experiment to be 1.1 μg. The results of the mouse challenge studies are shown in ***Figure B.3***. In summary, when followed in 4 hours with 40 ug LPS, 3.2 ug SEB and 1.6 ug SEB were 100% fatal, while 0.8 ug SEB only killed 1/6 of the mice. All the mice receiving lower doses of SEB survived and the controls for LPS and SEB alone all behaved as expected (all mice survived).

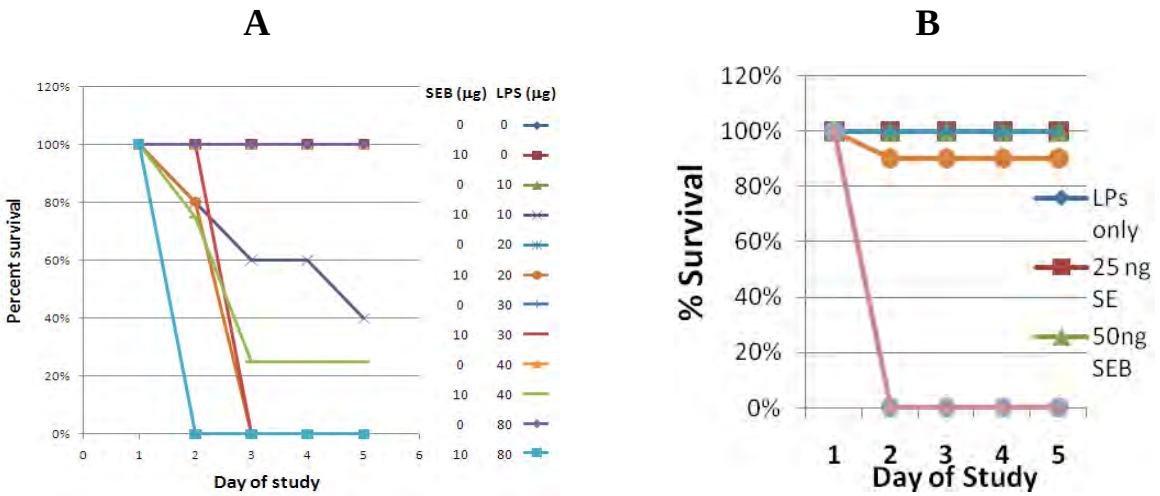

Figure B.3. Calibration of LPS and SEB in BALB/c mice. (A) Groups of 6 mice were challenged with SEB at 10 µg/mouse by intraperitoneal (IP) injection followed by IP injection of the indicated doses of LPS. Mortality was scored for up to 4 days. (B) Mice were injected with increasing doses of SEB followed by 40 µg LPS 4h later and mortality was scored for up to five days.

Phase I Objective 4: *Examine the inhibitory activity of IVIG and immune sera from monkeys on superantigenic activities of SEB and related toxins in vitro.*

For this objective, the toxin neutralizing activity (TNA) of the selected IVIG batches, monkey sera, and human plasma was determined. TNA assays were performed primarily for SEB, but also for other bacterial superantigens in several cases. Two types of experiments were performed.

- In the first type, the inhibitory activity of a constant concentration of IVIG or sera on SEB dose response was determined (An example of this type of experiment is shown in ***Figure B.2B***). In multiple experiments, 0.5mg/ml of IVIG was able to cause up to a thousand fold increase in SEB EC_{50} indicating significant neutralization/protection when compared to the samples assayed without IVIG. (Effective Concentration (EC) causing half maximal activation of the lymphocytes).
- In the second type of experiments, we determined the IC_{50} (inhibitory concentration resulting in 50% reduction in IFNγ release) by using a constant concentration of toxin and diluting the particular subset of IVIG samples assayed.

Table B.3 shows the IC_{50} values for inhibition of SEB and other tested toxins by the selected six IVIG samples. The inhibitory activity of monkey sera also was tested. However, due to limited volume of sera available, it was not possible to use these samples in the animal studies. Therefore, it was decided not to pursue the monkey sera.

IVIG#	SEB	SEA	SEC	SED	TSST-1	SpeA	SpeB	SpeC
J 16G171	31.85	191.8	97.86	163.6	27.24	33.49	4.09	16.33
J 29G280	7.34	114.5	71.50	14.28	34.08	30.52	5.23	12.41
I 42335	31.07	107.7	54.13	59.89	20.56	20.94	1.40	2.64
I 36291	18.09	110.3	92.05	109.67	8.93	12.54	0.98	21.65
I 28402	31.36	62.60	84.09	30.58	21.26	217.7	2.49	4.88
I 19135	51.38	109.9	42.47	42.83	20.33	55.85	7.40	8.36
Mean IC_{50}	28.52	116.2	73.68	70.15	22.07	61.84	3.60	11.04

Table B.3. IC50 values for inhibition of the indicated toxins by 6 IVIG

In order to produce positive and negative controls for efficacy studies, and to explore the cross reactivity of toxin specific antibodies against the other toxins, we performed affinity purification of anti-SEB antibodies from a pool of IVIG sample pools. SEB was coupled to AminoLink Plus Beads using Peirce Immobilization kit (cat #20394) according to the manufacturer's instructions. Elution was performed by sequential collection of ~600 ul fractions at pH 2.5. The pooled IVIG, the column flow-through (depleted of αSEB Abs), and the eluted purified/enhanced αSEB fraction were tested in both total Ig and αSEB ELISA assays to confirm the αSEB component before being used in toxin neutralization assays with PBMC, as described above. It was found that affinity purified antibody was close to 1000 times more potent in inhibiting SEB-induced IFNγ (***Figure B.4***). We then analyzed the inhibitory activity of purified anti-SEB toward other toxins. The anti-SEB antibody showed strong cross inhibition toward SEC and SPeA, and moderate inhibitory activity toward SEA and SED. In contrast, no significant inhibition of TSST-1 and SpeC was observed. These results are summarized in ***Table B.4***.

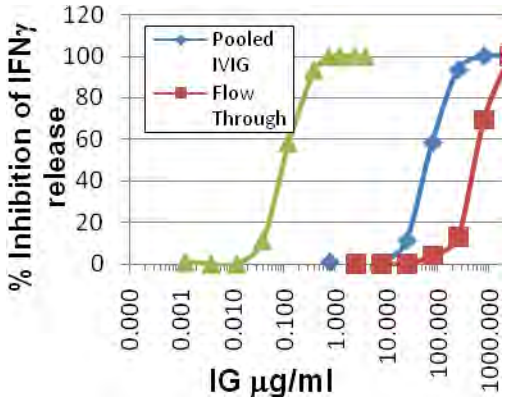

Figure B.4. Toxin Neutralizing Activity of purified anti-SEB antibodies (green triangles) vs the original pooled samples and column flow through (anti-SEB depleted).

Fraction	IC50 against individual toxins µg/ml						
	SEB	SEA	TSST-1	SEC	SED	SpeA	SpeC
Pooled IVIG	69.04	175.8	24.59	67.22	100.6	76.91	22.49
Flow Through	611.7	265.5	17.67	100.2	53.00	116.2	57.79
αSEB Fraction	0.11	1.68	>20	0.20	2.13	0.97	>20

Table B.4. IC_{50} values for IVIG, flow through and anti-SEB purified fraction

Phase I Objective 5: *Determine the toxin neutralizing activity (TNA) of IVIG and monkey immune sera in vivo using a mouse model of SEB-induced toxic shock.*

Toxin neutralizing activity of IVIG was determined in BALB/C mice as a proof of feasibility of an anti-SEB antibody therapeutic. We examined regular IVIG, affinity purified anti-SEB derived from IVIG, and flow through from the affinity column (αSEB depleted IVIG). SEB (1.5 LD_{50}) was incubated with the antibodies or saline and administered to groups of 6 BALB/C mice by intraperitoneal injection. Mice were then injected with 40 µg of LPS 4 hours later and observed for 96 hours. Data are shown in ***Figure B.5***. 50% of the mice

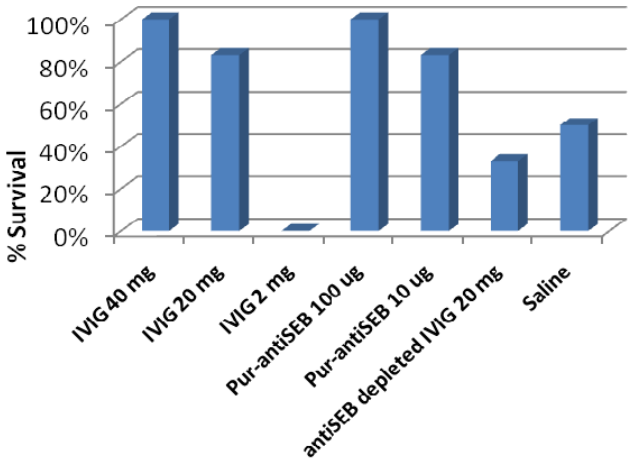

Figure B.5: *In vivo* efficacy of IVIG, purified anti-SEB and anti SEB-depleted human antibodies against lethal challenge in mice.

treated with no antibody died as expected with the indicated dose. While 20 mg of IVIG fully protected the mice, a dose of 2 mg was ineffective (as expected). Purified anti-SEB showed complete protection at a dose of 100 μg and 83% protection at 10 μg. IVIG depleted from anti-SEB did not provide any protection demonstrating that the protection is specific and mediated by anti-SEB antibodies in the IVIG. Taken together, these data indicate that human anti-SAg antibodies can provide protection *in vivo*, and that the titer of such antibodies in IVIG purified from the general naïve population is too low and an increase in the titer, as proposed here by immunization can increase its therapeutic potency.

Key Research Accomplishments

- Purified IVIG from pooled plasma samples were assayed for multiple toxin antibodies and found to contain a diverse range of titers

- 30 individual plasma samples were also assayed against the 8-toxin panel using newly developed ELISA techniques and the results were similarly diverse although more pronounced

- Cross reactivity profiles for the various toxins, SEA, SEB, SEC, SED, TSST-1, SPeA, SPeB, and SPeC were determined using both solid phase reactivity (ELISA) and *in vitro* (lymphocyte based) assays. In general, SEB showed strong cross reactivity with SEC and SpeA while moderate activity with SEA and SED.

- Assays based on lymphocyte activation showed that preparations that were enhanced for α-SEB Abs could inhibit lymphocyte activation over 1000 fold when compared to controls or a corresponding sample that had been depleted of α-SEB Abs.

- Demonstrated feasibility of treatment using high titer immunoglobulin in mouse model of toxic shock

Reportable Outcomes

- IBT intends to submit the results in form of a manuscript to a peer-review scientific journal. This manuscript is currently in preparation and expected to be submitted by August 2008.

- An NIH U01 grant application has been submitted in collaboration with Omrix Biopharmaceuticals and Brigham Womens' Hospital which is partly based on the results obtained from this study. The proposal seeks to identify high titer individuals as an alternative source of human immunoglobulin enriched in antibodies against all superantigens.

- SEB / LPS model for SEB toxicity in BALB/c mice – an LD50 of 1.1 ug of SEB when given four hours before a dose of 40 ug LPS using 4-6 week old female BALB/c mice.

- Poster presentation at the 2008 ASM Biodefense and Emerging Diseases Research Meeting held in Baltimore in February titled: "Therapeutic Human Hyperimmune Polyclonal Antibodies against Staphylococcal Enterotoxin B" (see appendix):

- Assays Developed:
  1.) *In Vitro* assays based on lymphocyte activation for the toxins SEA, SEB, SEC, SED, TSST-1, SPeA, SPeB, and SPeC with a solid phase, quantifiable, (ELISA) endpoint. These assays can also be used to determine the level of inhibition provided by any number of agents.
  2.) Protocols for separating and quantifying specific toxin Abs from a pool of purified immunoglobulin
  3.) Quantitative ELISA capture assays for α-toxin Abs using plasma samples, purified Ig, or even rhesus monkey plasma.

Conclusion

In summary, the Phase I SBIR project was successful in i) demonstrating the feasibility of a human hyperimmune polyclonal antibody strategy for neutralization of SEB toxicity, ii) developing standard assays for evaluation of such therapeutic in preclinical efficacy studies. It is anticipated that in Phase II a clinical therapeutic candidate will be developed for advance development. This therapeutic can be used both ofr prophylaxis and post-exposure treatment of warfighters and civilians in the case of a biowarfare attack with aerosolized SEB.

Appendices

Therapeutic Human Hyperimmune Polyclonal Antibodies against Staphylococcal Enterotoxin B

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Introduction

Background: *Staphylococcus aureus* is associated with numerous human diseases and there is a high incidence of strains that are resistant to commonly used antibiotics. Both community and hospital acquired infection rates are becoming a significant concern to public health. In addition, staphylococcal enterotoxins (SEs), primarily SEB, can be disseminated as a weaponized aerosol leading to incapacitation or death. This toxicity results from superantigenic activity which bypasses the normal MHC activation pathways of adaptive immunity. Based on evidence found to date in our lab and others, it seems apparent that high titer neutralizing antibodies represent the most promising therapeutic approach for treatment.
Methods: In order to demonstrate the feasibility of developing hyperimmune antibodies, in this study, purified IVIG samples from normal donors were tested by ELISA to identify samples with high titer against SEB as surrogates for hyperimmune serum. These samples were used initially to develop and standardize *in vitro* toxin neutralization assays based on T-cell proliferation and cytokine responses, as well as demonstration of therapeutic efficacy in a mouse model of toxic shock. IBT's cGMP produced recombinant SEB vaccine (STEBVax) will enter clinical trial in Spring 2008. Protocols will be developed for plasmapheresis of vaccinated individuals to prepare hyperimmune serum.
Results: Initial results indicate that IVIG samples can be identified with high titer anti-SEB antibody. Data from *in vitro* and *in vivo* toxin neutralizing activity of these samples will be presented.
Conclusions: Preliminary findings support the notion that human antibody therapeutics can be developed to treat SEB-induced toxic shock.

Background

SEB toxicity results from its superantigenic activity. As a bacterial superantigen (SAg) SEB binds to human major histocompatibility complex (MHC) class II on antigen presenting cells (APC), cross-links MHC to the T-cell receptor (TCR) leading to polyclonal activation of T-lymphocytes (***Figure 1 and 2B***). Interactions of superantigens with host cells differ from conventional antigens (***Figure 2A***), in that SAgs: 1) directly bind outside the peptide-binding groove of MHC class II; 2) and exert biological effects without internalization or "proteolytic processing" by APC; and 3) are not MHC-restricted. As a result, superantigens stimulate both APC and T cells resulting in activated T cells that massively proliferate and produce large quantities of pro-inflammatory cytokines in particular IFN-γ, which further activates APCs (***Figure 1***). Release of IL-1 and TNF-α leads to fever as well as capillary leak culminating in hypotension and systemic shock resulting in death (Figure 1). Superantigens are also known to increase the host susceptibility to endotoxin. Superantigens also cause T cell apoptosis and/or anergy thus allowing for the pathogen to evade the adaptive immune response (***Figure 1***).
Based on compelling evidence emerging from our prior studies and those reported by others, it is evident that neutralizing antibodies represent the most promising therapeutic approach for treatment of SE intoxications. Furthermore, there is strong evidence that polyclonal antibodies against one toxin can provide partial or full protection against certain other Ses. Based on these findings this project was designed to develop polyclonal human antibodies against SEB (Figure 2).

Figure 1: Cascade of toxic events resulting from release of superantigens in the bloodstream.

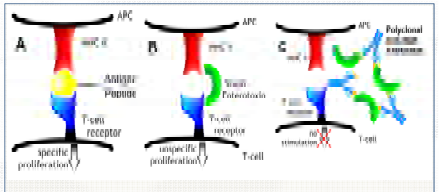

Figure 2: Interaction of TCR and MHC loaded with antigenic peptide during the normal T cell activation (A) and during superantigenic activation by staphylococcal enterotoxins (B). The latter can be inhibited by polyclonal antibodies such as anti-SEB HIS as shown in (C).

Study Objectives

1. Establish cell based and in vivo assays for determination of the potency of human anti-SEB antibodies.
2. Use IVIG and high titer human plasma as surrogates for anti-SEB hyperimmmune serum for proof of feasibility.
3. Complete regulatory documentations for a recombinant SEB vaccine (STEBVax) and file IND with FDA.
4. Conduct a Phase I Clinical Trial to determine the safety and immunogenicity of STEBVax.
5. Design and implement plasmapheresis trials in healthy individuals vacccinated with STEBVax.
6. Purify IgG from vaccinated volunteers.
7. Complete pre-clinical efficacy studies for hyperimmmune IVIG in mice and non human primates.

Anti-SEB toxin neutralization activity of IVIG samples

In order to functionally characterize the IVIG samples, two toxin neutralization assays were established. In both assays SEB-induced release of IFN-γ in PBMC is used as readout. In the first type of assay a constant concentration of SEB (5 ng/ml) is pre-incubated with serial dilutions of IVIG before adding to PBMC. This assay can be used to determine IC_{50} (50% inhibitory concentration) for IVIG samples. ***Figure 3*** shows representative data for 3 IVIG samples. Individual IC_{50} values are also shown for all the IVIG samples tested to date in ***Table 2***.
In the second type of assay a constant dilution of the IVIG (1:200; 250 µg/ml) is pre-incubated with serial dilutions of SEB. In this assay the EC_{50} (50% effective concentration) for SEB can be determined in the presence or absence of IVIG. EC_{50} ratio, defined as the ratio of the SEB EC_{50} in presence and absence of IVIG, can be used as a measure of the ability of the antibody to decrease sensitivity of the cells to SEB. ***Figure 4*** shows representative data for 3 IVIG samples. Individual EC_{50} values are also shown for all the IVIG samples tested to date in ***Table 2***.
To ensure that the inhibitory effect of the IVIG is specific to SEB, the effect of two IVIG samples on phytohemagglutinin (PHA) induced activation of PBMC was examined. As shown in ***Figure 5***, IVIG had no inhibitory effect on PHA-induced IFN-γ in PBMC. These data demonstrate that the inhibitory activity of IVIG relates to anti-SEB antibody content.

IVIG #	IC_{50} (µg/ml)
J 16G171	438.9
J 02G021	469.9
K 15G191	356.8
J 15G165	389.0
I 42335	182.1
I 18121	403.8
I 36291	205.3

IVIG #	EC_{50} Ratio
J 16G171	374.63
J 02G021	636.88
J 15G165	1521.88

Table 2: IC_{50} values for individual IVIGs and changes in SEB EC50 values in presence of IVIG samples.

IVIG #	α-SEA µg/ml	α-SEA µg/g IVIG	IVIG #	α-SEA µg/ml	α-SEA µg/g IVIG
J 16G171	4.44	88.72	I46365	4.97	99.45
J 12G135	4.66	93.30	I 40325	4.20	84.05
J 24G231	3.39	67.80	I 23151	4.43	88.66
J 29G280	4.54	90.71	I 29221	4.40	87.94
J 10G105	3.23	64.54	I 17112	4.86	97.23
J 02G021	4.51	90.14	I 38311	3.60	72.06
J 15G165	5.46	109.11	I 36291	4.20	83.93
K 15G191	4.40	88.00	I 28402	4.59	91.85
I 42335	5.09	101.80	I 31241	3.95	79.06
I 37305	4.32	86.45	I 19135	4.15	83.07
I 18121	3.71	74.14	I 49395	5.48	109.56
I 151411	3.76	75.19			

Table 3: anti-SEA ELISA titer of IVIG samples.

Toxin	antibody content µg/ml	antibody content µg/g IVIG
SEA	**3.96**	**79.18**
SEC-1	**36.87**	**737.49**
SED	**1.51**	**30.29**
TSST-1	**11.26**	**225.21**
SPEA	**20.76**	**415.22**
SPEB	**4.06**	**81.20**
SPEC	**16.49**	**329.76**

Table 4: ELISA titer of IVIG samples against multiple staphylococcal enterotoxins, toxic shock syndrome toxin 1 (TSST-1) and streptococcal pyrogenic exotoxins (SpeA, SpeB, and SpeC).

Reactivity of IVIG with other staphylococcal and streptococcal superantigens

We further investigated if IVIG contains specific antibodies against other bacterial superantigens. Initially we determined the anti-SEA titer of 23 IVIG Samples. All the samples contained measurable anti-SEA antibodies. The data are shown in ***Table 3***. We also measured the antibody reactivity of one IVIG sample toward other staphylococcal toxins including SEC-1, SED, TSST-1, as well as streptococcal pyrogenic exotoxins SpeA, SpeB, and SpeC. As shown in table 4, antibodies to all these toxins could be readily detected in IVIG. Highest level of antibody was found against SEC-1 and lowest antibody content was for SED.

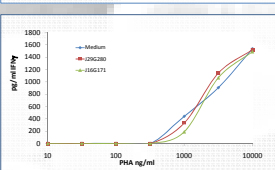

Figure 5: IVIG do not inhibit the activation of T cells by PHA. Cells were stimulated with PHA (10-10000 ng/ml) in presence or absence of 250 µg/ml IVIG and IFN-γ production was measured 48h later.

Human IVIG samples contain low to moderate amounts of anti-SEB antibodies.

28 IVIG samples from Omrix Biopharmaceuticals (50 mg/ml) were titered for anti-SEB antibody content using specific ELISA. Anti-SEB antibodies were captured on STEBVax-coated plates. To generate a standard curve, different concentrations of human IgG were captured on goat-anti human IgG. Anti-SEB content of IVIG samples was then determined using this standard curve. The data for the 28 IVIG samples are shown in ***Table 1***.

IVIG #	α-SEB µg/ml	α-SEB µg/g IVIG	IVIG #	α-SEB µg/ml	α-SEB µg/g IVIG
J 16G171	7.61	152.30	I 18121	5.89	117.77
J 12G135	6.07	121.31	I 151411	5.51	110.16
J 24G231	5.84	116.87	I46365	5.95	119.03
J 29G280	9.41	188.24	I 40325	4.54	90.77
J 10G105	5.41	108.11	I 23151	6.71	134.14
J 02G021	6.31	126.17	I 29221	6.02	120.36
J 15G165	6.27	125.42	I 17112	7.25	144.90
K 15G191	5.24	104.76	I 38311	5.70	114.00
I 42335	4.08	81.52	I 36291	5.87	117.46
I 32255	6.43	128.56	I 28402	7.03	140.68
I 30231	6.46	129.28	I 31241	5.69	113.72
I 48385	6.36	127.21	I 19135	5.19	103.77
I 44345	6.12	122.32	I 47371	6.61	132.15
I 37305	6.23	124.61	I 49395	6.18	123.54

Table 1: Anti-SEB content of human IVIG samples

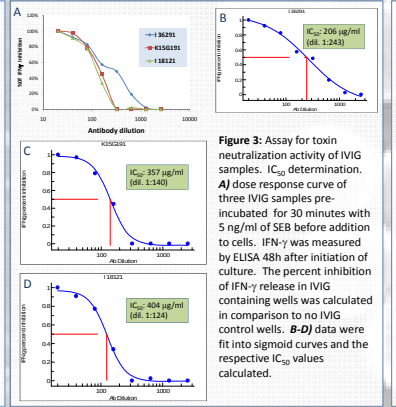

Figure 3: Assay for toxin neutralization activity of IVIG samples. IC_{50} determination. ***A)*** dose response curve of three IVIG samples pre-incubated for 30 minutes with 5 ng/ml of SEB before addition to cells. IFN-γ was measured by ELISA 48h after initiation of culture. The percent inhibition of IFN-γ release in IVIG containing wells was calculated in comparison to no IVIG control wells. ***B-D)*** data were fit into sigmoid curves and the respective IC_{50} values calculated.

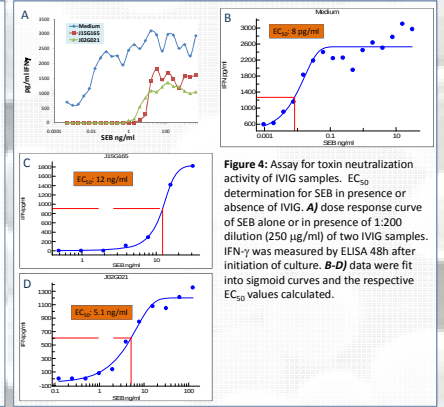

Figure 4: Assay for toxin neutralization activity of IVIG samples. EC_{50} determination for SEB in presence or absence of IVIG. ***A)*** dose response curve of SEB alone or in presence of 1:200 dilution (250 µg/ml) of two IVIG samples. IFN-γ was measured by ELISA 48h after initiation of culture. ***B-D)*** data were fit into sigmoid curves and the respective EC_{50} values calculated.

Summary and conclusion

- IVIG contain low to moderate levels of antibodies reactive to a wide range of staphylococcal and streptococcal exotoxins.
- Anti-SEB antibodies in IVIG can potently inhibit the toxic effects of SEB.

Future direction and studies in progress

- Complete toxin neutralization studies of IVIG with other toxins (in progress).
- Demonstration of *in vivo* neutralization activity f IVIG in mouse model of toxic shock (in progress).
- Affinity purification of anti-SEB antibodies from IVIG (In progress).
- IND submission for STEBVax (in progress).
- Phase I clinical trial for STEBVax (Spring 2008).
- Plasmapheresis trials and production of hyperimmmune Ig (2009).
- Animal efficacy studies of anti-SEB hyperimmmune Ig (2009).

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